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Differences in LTM-forming capability between geographically different strains of Alberta *Lymnaea stagnalis* are maintained whether they are trained in the lab or in the wild

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SUMMARY

We found strain differences in the ability of wild Alberta *Lymnaea stagnalis* to form long-term memory (LTM) following operant conditioning when *L. stagnalis* were collected from the wild and trained in the laboratory. *Lymnaea stagnalis* obtained from the Belly River watershed had an enhanced ability to form LTM compared with those from an isolated pond (referred to as Jackson snails). We therefore asked whether the differences in cognitive ability were an epiphenomenon as a result of training in the laboratory. To answer this question we trained each specific strain (Belly and Jackson) in both the laboratory and the field (i.e. in their home pond and in the pond where the other strain resided – referred to as the visitor pond). We found that within each strain there was no difference in the LTM phenotype whether they were trained in the lab or in either their home or visitor pond. That is, the strain differences in the ability to form LTM were still present. Interestingly, we found no strain differences in the ability to learn or the ability to form intermediate-term memory (ITM).

Key words: *Lymnaea*, operant conditioning, long-term memory, strain differences, laboratory *vs* wild conditions.

INTRODUCTION

Differences in cognitive ability have been noted previously between strains of the same species of rodent (i.e. within-species variation) (Nguyen et al., 2006). In fact, differences in cognitive ability within the same strain of a species have also been observed that are dependent on both the laboratory performing the experiment and the personnel within each laboratory (Crabbe et al., 1999; Whalsten et al., 2006). There is also a long history of attempting to create specific stable within-strain differences in cognitive ability by the selective mating of 'smart' (e.g. maze bright) *vs* 'not so smart' (e.g. maze dull) individuals of the species (Tolman, 1924; Tyron, 1931). Finally, it is believed that outbred members of the same species, e.g. *Rattus norvegicus*, or strains that are more closely related to the non-domesticated rat (i.e. more wild-like such as the Long–Evans variety) have fewer cognitive deficits compared with inbred rats, more distantly related to the wild-type (Harker and Whishaw, 2002). It is unclear at present what the causes of these within and between strain differences are or whether in fact they actually exist, as some of the apparent differences are not observed in different learning and memory tasks (Brush, 2003). The lack of understanding of why on specific tasks some rodent strains perform better than others is due to the complexities of the behavioural task and the complexity of the mammalian brain. In our model system, the pond snail *Lymnaea stagnalis* (L.) where a single neuron is known to be a necessary site for long-term memory (LTM) formation (Scheibenstock et al., 2002; Lukowiak et al., 2008), we have also found within-species variation in the ability to form LTM following operant conditioning of aerial respiratory behaviour (Orr et al., 2008; Orr et al., 2009).

In *L. stagnalis* geographically separate strains (The Netherlands *vs* Alberta Belly River snails) have different cognitive abilities (Orr et al., 2008). That is, laboratory-reared snails (over 250 generations; originally derived from snails collected in the Province of Utrecht in The Netherlands) had a reduced ability to form LTM compared with snails collected from the Belly River drainage (i.e. Belly snails) in Southern Alberta. Initially the differences in LTM-forming ability between the lab-reared and the Belly snails were hypothesized to be due to the 'wild' snails having an enriched environment compared with the lab-reared snails. However, that hypothesis was rejected because: (1) Belly snails hatched in the lab from eggs collected in the wild still exhibited enhanced LTM formation compared with the lab-reared snails; and (2) snails freshly collected in the same area of The Netherlands where the founding members of the lab colony were originally collected exhibited comparable LTM-forming abilities to their lab-reared descendants (i.e. not as good as Belly snails). Thus, we concluded that the Belly snails had an enhanced ability compared with the Dutch snails to form LTM following operant conditioning of aerial respiration [i.e. an inherent strain difference (Orr et al., 2008)]. Because the Belly River ponds are located over 200 km from our Calgary laboratory, we sought a closer collection site (in order to in part reduce our 'carbon footprint') that had an abundance of *L. stagnalis*. We found such a site (only a few kilometres from the Lukowiak residence) and collected wild *L. stagnalis* from this new location, the Jackson pond (referred to as Jackson snails). However, we found in our initial experiments that the Jackson snails did not possess the enhanced memory-forming capabilities seen in the Belly snails but rather exhibited LTM-forming abilities more akin to the Dutch wild and lab-reared snails. More recently, we showed that we could enhance the memory-forming capabilities of the Jackson snails when trained in the lab by exposing them to the scent of a sympatric predator, the Tiger salamander [salamander effluent, SE (Orr et al., 2009; Orr and Lukowiak, 2010)]. We wondered, however, whether the differences in cognitive ability between the two strains of Alberta

snails were due to the stress of training in the laboratory, as too much stress in *L. stagnalis* blocks LTM formation (Martens et al., 2007b).

A plausible and testable hypothesis to explain the difference in the ability to form LTM between Belly and Jackson snails is that testing in the laboratory causes more stress in the Jackson snails (i.e. they are less adaptable to lab conditions), resulting in a diminished ability to form LTM. We have recently demonstrated that an environmental stressor, crowding, prevents or worsens LTM formation (de Caigny and Lukowiak, 2008a; de Caigny and Lukowiak, 2008b). To test our hypothesis we trained Belly and Jackson snails both in the laboratory and in the field. We found, however, that under these conditions Jackson snails have an inferior ability to form LTM compared with the Belly snails, but interestingly enough Jackson snails formed intermediate-term memory (ITM) as well as the Belly snails. This suggests to us that while the acquisition of the new skill (i.e. learning) and the ability to form ITM are similar in the two strains, it is the next step in the formation of LTM, the one requiring altered gene activity, where the strain difference lies. The cause of this strain difference is not understood.

MATERIALS AND METHODS

Snails

Lymnaea stagnalis (L.) is a cosmopolitan species found worldwide in temperate regions. In this investigation we utilized two geographically distinct wild populations of snails in Southern Alberta: (1) snails collected from six seasonally isolated ponds in the Belly River drainage, Canada (referred to as Belly snails; latitude: 490 31′ N; longitude: 1130 16′ W and elevation: 961 m) and (2) snails from a 20 year artificial dugout pond (referred to as Jackson snails; latitude: 500 44′ N; longitude: 1140 23′ W and elevation: 1254 m); the pond has no obvious water inlet or outlet and is filled by snow melt and rain accumulation.

All collected snails were *L. stagnalis*. They were identified using the keys of Clarke (Clarke, 1981) and Clifford (Clifford, 1991), as well as descriptions from published works in similar localities in Alberta (Boag and Pearlstone, 1979; Boag et al., 1984). We do not know when or how *L. stagnalis* was introduced to the 20+ year old Jackson pond. However, it is clear that snails can ‘migrate’ long distances on the feet of waterfowl (Gittenberger et al., 2006).

This study began during spring 2006 with pilot experiments, continued into autumn 2007 and finished in July 2009. Snails from the Belly River ponds and the Jackson pond were collected from several random locations within 2 m of the shoreline of the ponds using dip nets. Before operant conditioning training (see below) snails were maintained for no more than a few days in the pond (either the ‘home’ pond or the ‘visiting’ pond in fixed nets such that water and other small pond debris could freely move through but the snails could not escape. This was done because we had to transport the snails over a distance of approximately 200 km and they were most likely in a somewhat ‘stressed’ state due to the effects of the journey. We tried to make sure that the person who would train the snails during a specific week did not know the origin of the snails, but this proved to be very difficult. Thus, we cannot emphatically state that the experiments in the field were performed blind. However, the experiments in the laboratory setting were performed in a blind manner as the investigators (K.S.L. and J.H.) performing the experiments in the lab did not collect the snails they were training nor were they told where the snails came from until after the completion of the studies.

Aerial respiratory behaviour

Lymnaea stagnalis are bimodal breathers obtaining oxygen either through cutaneous respiration (i.e. directly through the skin) or through aerial respiration *via* a lung [i.e. gas exchange with the atmosphere (see Orr and Lukowiak, 2008; Orr and Lukowiak, 2010)]. To perform aerial respiration, the snail must surface and open its pneumostome (respiratory orifice) while contracting and relaxing the appropriate respiratory muscles. For a more detailed description see Lukowiak et al. (Lukowiak et al., 2003a). Aerial respiratory behaviour is driven by a three-neuron central pattern generator whose sufficiency and necessity have been demonstrated (Syed et al., 1990; Syed et al., 1992b). To increase aerial respiratory behaviour we made the pond water hypoxic (P_{O_2}<7 Torr, ~931 Pa) by bubbling N_2 through the training beaker for 20 min before the introduction of snails.

Operant conditioning

In the wild

In the wild snails were removed from their temporary holding aquaria in their home or visiting pond (either one of the Belly ponds or the Jackson pond). They were maintained in the natural setting until we had a sufficient number of snails to train. Thus, Jackson snails were maintained at the Belly river site (i.e. the visiting pond) and Belly snails were maintained at the Jackson site prior to training in the wild.

Both in the lab and in the field snails were placed into a 1 litre beaker containing 500 ml of hypoxic water (P_{O_2}<7 Torr, see above) taken directly from the pond (real PW). The animals were given a 10 min acclimatization period prior to the 30 min training session. By subjecting snails to a hypoxic challenge, the animals increase their rate of aerial respiration (Lukowiak et al., 1996; Lukowiak et al., 1998). The animals were operantly conditioned by applying a gentle tactile stimulus with a sharpened wooden applicator to their pneumostome as it began to open. The stimulus was strong enough to cause the snails to close the pneumostome yet gentle enough that the snails did not perform the full body withdrawal response. The contingent stimulation was given during both the training session (TS1) and the test for memory (MT). This pneumostome closer response is a graded part of the whole-snail escape response (Inoue et al., 1996). Every time the snail opened its pneumostome and received the stimulus during the training period, the time was recorded for future use in yoked control experiments. Yoked controls (see below) were performed for all behavioural experiments.

The operant conditioning procedure we utilized consisted of a single 0.5 h TS1 after which the snails were returned to their home aquaria (Haney and Lukowiak, 2001; Sangha et al., 2003a). The snails were then tested for memory (MT; i.e. a ‘savings test’) using a similar test to that of the training session. The time of the MT or recording is indicated as time after the TS1. Each operant conditioning experiment was replicated at least twice by utilizing two separate naive cohorts of 10–14 snails in the initial 0.5 h TS1 for each experiment.

Yoked control experiments

During the training period, yoked control snails received exactly the same number and sequence of stimuli as those of the operant conditioning group, but the stimuli were not contingent upon their pneumostome opening. However, these yoked control snails did receive a contingent stimulus to the pneumostome during the savings test session (MT). Snails that received yoked training were treated in an identical manner to that outlined in the ‘yoked operant conditioning procedure’ used previously (Lukowiak et al., 1996;

Lukowiak et al., 1998; Lukowiak et al., 2000; Lukowiak et al., 2003a; Lukowiak et al., 2003b; Lukowiak et al., 2008).

In the laboratory

The exact same training procedure as described above for training in the wild was used on wild snails in the lab except that snails were (1) maintained in aquaria on a diet of Romaine lettuce and spinach; (2) maintained in aquaria filled with artificial pond water (aPW); and (3) trained 'blindly' in aPW. Wild snails were acclimated to lab conditions for at least 4 days before training occurred. aPW was made from de-ionized water to which Instant Ocean sea-salts were added ($0.26\,g\,l^{-1}$).

Assignment of 'marks' to individual snails

Lymnaea stagnalis were given grades on an individual basis to show how well (or how poorly) they learned. The following grading scheme was used to assess learning: a snail that showed a 50% or greater reduction in attempted pneumostome openings from the first training session (TS1) to the memory test session (MT) was given an A, B was a 35–49.99% reduction, C was a 20–34.99% reduction, and F was assigned when a reduction of less than 20% was observed. This marking scheme has been successfully used before (e.g. Lukowiak et al., 2003a; Rosenegger et al., 2005).

Statistics

We analysed operant conditioning effects on snail behavioural data with repeated measures analysis of variance (ANOVA) where the within-subject factor of population was used and the between-subject factor of interval (time) was used. We used a Bonferroni multiple comparison test for *post-hoc* analysis. All repeated measures data were tested for equal variance using Mauchly's test for sphericity. In cases where sphericity could not be assumed, we used the conservative adjusted Greenhouse–Geisser *P*-values. For comparing snail 'marks' the Chi-square statistic was used. All statistics were performed on SPSS version 11.0.4 for Macintosh.

RESULTS

We first compared the ability of the two geographically distinct populations of Alberta *L. stagnalis* (Belly *vs* Jackson) to form LTM following a single 0.5 h training session in the laboratory. We found (Fig. 1, top) that snails collected from the Jackson pond, maintained and tested under laboratory conditions did not form LTM following the single 0.5 h TS1. These snails could, however, form ITM. That is, when tested 3 h after training (3 h MT) memory was present in that the number of attempted pneumostome openings was statistically fewer than in TS1 ($P<0.01$). However, when tested 24 h after TS1, memory was not present as the number of attempted pneumostome openings was not statistically different from that in TS1. In addition LTM was not observed in the yoked control snails. These data are similar to data obtained using lab-reared snails of a colony that was set up from snails collected from polders in the Province of Utrecht in the 1950s (Orr et al., 2007; Orr and Lukowiak, 2008) and from freshly collected snails from this same polder (Orr et al., 2008).

On the other hand, when we trained Belly snails (Fig. 1, bottom) that were maintained and tested in the lab with the same training procedure (0.5 h TS1) as was used with the Jackson snails we found that these snails had an enhanced ability to form LTM. In these snails the single 0.5 h TS1 resulted in an LTM that persisted up to 72 h. That is, the number of attempted pneumostome openings in the MT 72 h after TS1 was significantly smaller than the number in TS1 ($P<0.01$). However, memory was not seen when MT was performed 1 week after TS1. Thus, memory was present 3, 24 and 72 h after the single training session. Again, Belly snails subjected to the yoked control training procedure did not exhibit LTM 24 or 72 h after training. We conclude that in laboratory testing Belly snails have an enhanced ability to form LTM compared with the Jackson snails.

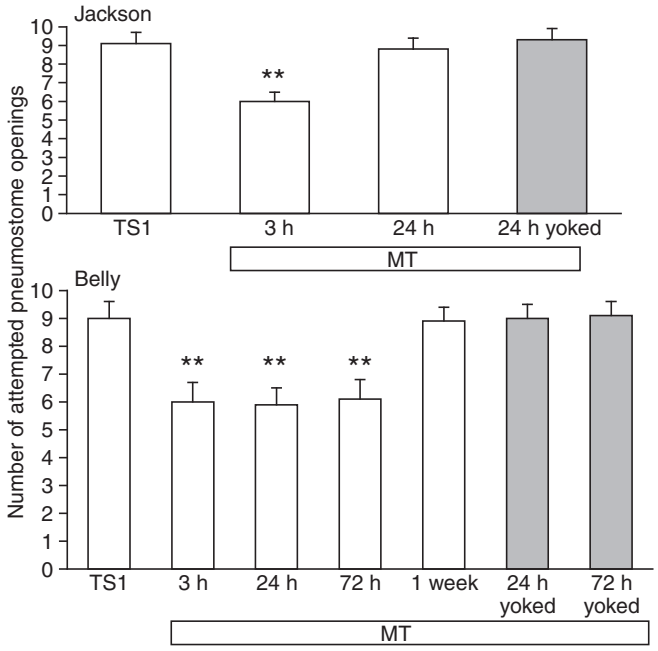

Fig. 1. Jackson and Belly snails: learning, intermediate-term memory (ITM) and long-term memory (LTM) when trained and tested in the laboratory. Top, four separate groups of snails were freshly collected from the Jackson pond and brought into the laboratory before operant conditioning training (a single 0.5 h training session, TS1). A naive cohort (N=23) was tested 3 h after TS1 and showed memory, i.e. 3 h MT was significantly less (**$P<0.01$) than TS1. A second cohort (N=40) was tested 24 h after TS1 and did not exhibit memory, i.e. 24 h MT was not significantly different ($P>0.05$) from TS1. A third cohort was the yoked control (N=40) and this group also did not exhibit LTM when tested 24 h after receiving the yoked training ($P>0.05$). Bottom, eight separate cohorts of snails were freshly collected from the Belly River drainage and brought into the laboratory before operant conditioning training (a single 0.5 h training session, TS1). A cohort of snails (N=31) was tested 3 h after TS1 and demonstrated memory, i.e. 3 h MT was significantly less (**$P<0.01$) than TS1. In a similar manner two separate cohorts of naive snails when tested 24 h (N=54) and 72 h (N=42) after TS1 also demonstrated LTM, i.e. both 24 h MT and 72 h MT were significantly less (**$P<0.01$ in both cases) than TS1. On the other hand a cohort of naive snails that was tested 1 week (N=29) following TS1 did not demonstrate LTM, i.e. 1 week MT was not significantly different ($P>0.05$) from TS1. Finally, neither the 24 h (N=49) nor the 72 h (N=40) yoked control cohort demonstrated LTM (i.e. 24 h yoke and 72 h yoke were not significantly different from TS1; $P>0.05$ in both cases).

We thought it was possible that the difference in memory-forming capability between the two populations of Alberta *Lymnaea* could be due to differences in acclimatization to a laboratory environment (e.g. the aPW or lab diet of lettuce affected the snails differently) between the two strains. That is, the Jackson snails may have a more difficult time than Belly snails adjusting to the laboratory environment. To test this hypothesis we tested each population in the wild both in their home pond and in the 'visiting' pond. Specifically, the snails were collected and tested in a natural setting eating real pond food and in real pond water. These data are presented in Fig. 2 (Jackson snails) and Fig. 3 (Belly snails). The strain differences in memory-forming

ability can still be clearly seen. That is, Jackson snails (Fig. 2) only had the ability to form ITM (memory at 3 h) and not LTM (no memory at 24 h) whether they were tested in their home pond (Fig. 2A) or the visiting pond (Fig. 2B). On the other hand, the Belly snails (Fig. 3) formed LTM that persisted for up to 72 h whether they were tested in their home pond (Fig. 3A) or the visiting pond (Fig. 3B). In these two geographically separate populations, in both the home and visiting pond LTM was not present in the yoked control preparations. Thus, we conclude that as a population Belly snails have a greater capacity to form LTM than do Jackson snails whether tested in the lab or in the wild.

The data presented in Figs 1–3 are group data. Another way of looking at the differences between the strains is to examine individual data for each trained snail. To accomplish this each snail was given a 'mark' (see Materials and methods) for its individual ability to form LTM. We compared the marks in each cohort 24 h after TS1 (Fig. 4). When we compared the marks given to the Jackson snails we found that there was no difference in the grade distribution ($P>0.05$) between snails trained in the lab and those trained in the wild (in their home pond). The data show that some of these snails do have the ability to form grade A memory (23–25%). However, the vast majority of these snails (63–64%) received an F grade. That is, over 60% of these snails had an inability to form LTM. In contrast, Belly snails (in either the lab or home pond condition) had a significantly increased ability to form grade A learning (34–38%) and relatively few (35–40%) received a F grade. Importantly, when we compared A–C (i.e. 'passing') grades the results were even more dramatic. In Belly snails over 60% receive a passing grade whilst in Jackson snails only 36% receive a passing grade (i.e. about 2/3 failed).

Finally we compared the response of the two different strains in the training session (TS1) and in the 3 h memory test (3 h MT) to determine whether the two strains also differed in the ability to acquire new information (i.e. learn) or form ITM. We first examined within-strain responses. In the Jackson snails there was statistically no difference in the number of attempted pneumostome openings in TS1 in lab *vs* field (i.e. home pond) experiments ($P=0.9$, $N=30$). In addition there was also no statistical difference in the 3 h memory between field and laboratory ($P<0.645$, $N=20$). Similar results were obtained with Belly snails. There was no significant difference in TS1 between lab and field ($P=0.874$, $N=48$) and in the 3 h memory test ($P<0.7$, $N=21$).

We then tested between strain responses for TS1 and the 3 h MT for the results obtained both in the lab and in the field (i.e. home

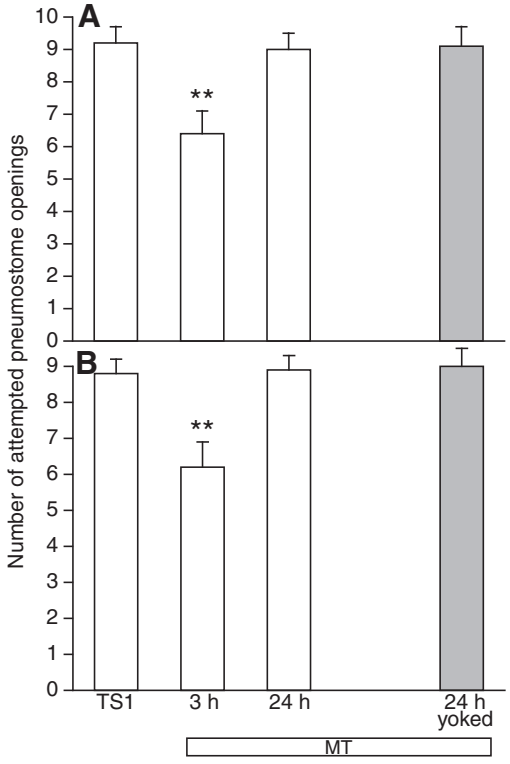

Fig. 2. Jackson snails exhibit ITM but not LTM. Jackson snails were trained with a single 0.5 h training session either in their home (A) or in a visiting (B) pond. They performed the same as the Jackson snails in the lab with the same training procedure. Thus, ITM was observed whilst LTM was not. That is, the number of attempted openings 3 h after TS1 was significantly less (**$P<0.01$, $N=20$ in the home pond and **$P<0.01$, $N=22$ in the visiting pond) than TS1. However, LTM was not observed (24 h MT was not statistically different from TS1; $P>0.05$, $N=32$ home pond and $P>0.05$, $N=25$ visiting pond). Finally the yoked control cohorts also did not demonstrate LTM ($P>0.05$, $N=28$, home pond; $P>0.05$, $N=18$, visiting pond).

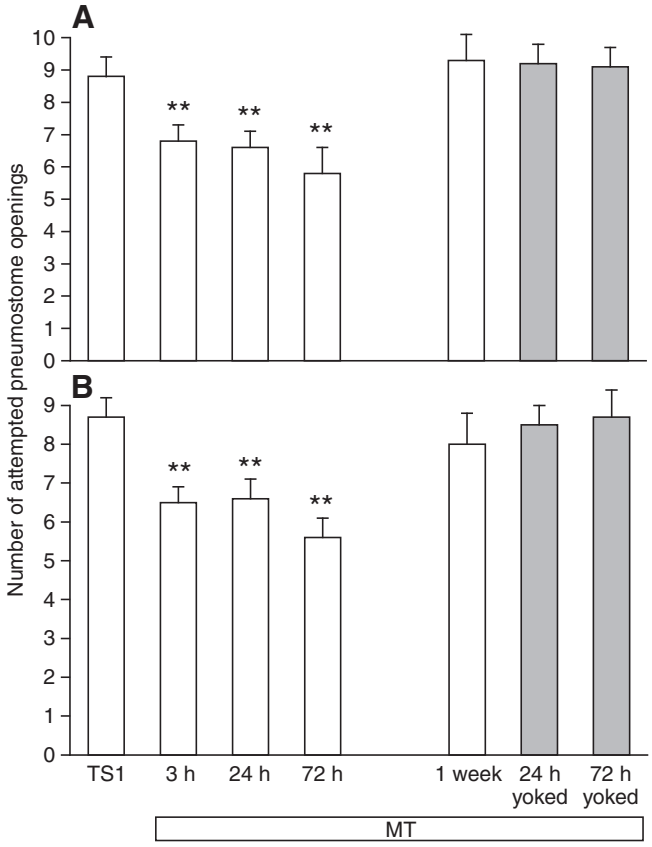

Fig. 3. Belly snails exhibit LTM in both their home (A) and visiting (B) pond following a single 0.5 h training session. Belly snails exhibit both ITM 3 h after training (**$P<0.01$, $N=25$, home pond; and **$P<0.01$, $N=20$, visiting pond) and LTM 24 h (**$P<0.01$, $N=53$, home pond; and **$P<0.01$, $N=32$, visiting pond) and 72 h (**$P<0.01$, $N=23$, home pond; and **$P<0.01$, $N=21$, visiting pond) after TS1. However, they did not exhibit LTM 1 week ($P>0.05$, $N=32$, home pond; and $P>0.05$, $N=15$, visiting pond) later nor did they exhibit LTM in the four yoked control cohorts (24 h, $P>0.05$, $N=26$; and 72 h, $P>0.05$, $N=25$, home pond; 24 h, $P>0.05$, $N=24$; and 72 h, $P>0.05$, $N=20$, visiting pond).

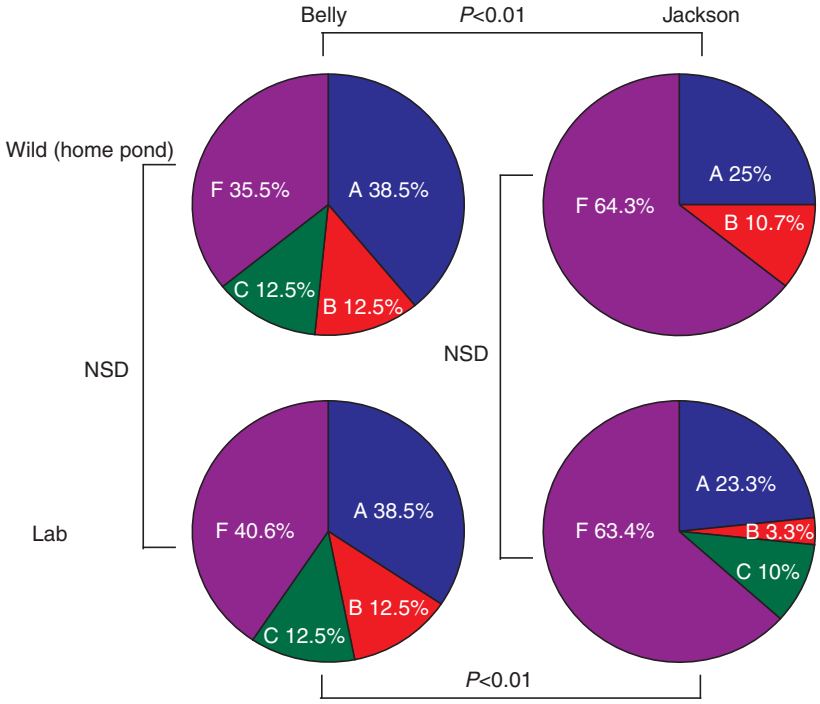

Fig. 4. Individual marks for each Jackson and Belly snail trained in the lab and in the field. The data are from the same snails as plotted in Fig. 1, and from the 'home' snails in Figs 2 and 3. There was no statistical difference (NSD) in the grade distribution between the two Jackson cohorts examined 24 h after TS1 (Chi-squared analysis; P=0.976, N=72). Likewise there was no statistical difference in grade distribution within the Belly snails dependent on where they were trained and tested (Chi-squared analysis; P=0.931, N=107). However, the grade distributions differed significantly between the Jackson and Belly snails under all conditions. That is, Belly snails received more A grades and fewer F grades than Jackson snails however the comparisons were made (Chi-squared analysis; P<0.01 for both comparisons).

pond). There was no statistical difference in the attempted number of pneumostome openings in TS1 between the Jackson and Belly snails under either the laboratory (P=0.435, N=30) or the 'wild' condition (P=0.487, N=32). Since there was no difference in TS1 we asked whether there was any statistical difference in the 3 h memory between the Jackson and Belly strains in the lab and in the wild (home pond). We found that there was no statistical difference in the number of attempted openings in the 3 h MT (lab: P=0.767, N=21; field: P=0.342, N=20). Thus, we conclude that the two strains are equally capable of associative learning and equally capable of forming ITM.

DISCUSSION

The data presented here show: (1) clear differences in the ability of two spatially distinct (~200 km) populations of Alberta *Lymnaea stagnalis* to form LTM; and (2) that this difference in LTM-forming ability is not due to a difference in adaptability to a lab environment between the two populations. That is, the Jackson snails were not in a more stressful situation compared with the Belly snails when they were trained in the lab. The differential LTM-forming ability was seen irrespective of whether snails were trained in the lab or in the wild (either in their home pond or in the visiting pond). Moreover, this difference in cognitive ability was seen both in the group data and when individual 'marks' for each snail were examined. However, a difference in learning ability and the ability to form ITM was not found between the two groups of snails. It is unclear to us why this difference in memory-forming capability between strains arose and what maintains it. It is also clear from the data that as regards the ability of Belly snails to form LTM, the training and testing of wild snails in the lab does not result in a decreased capability to from LTM compared with training and testing in the 'wild' (either in the home or the visiting pond).

Strain-related differences in cognitive ability have previously been seen in both vertebrates and invertebrates (Orr et al., 2008b; Orr et al., 2009; Ings et al., 2005; Brush, 2003; Tyron, 1931; Tolman, 1920). Moreover, even within the same strain differences in cognitive performances have been noted due to differences in factors such as the specific laboratory environment (Crabbe et al., 1999; Wahlsten et al., 2006). There have also been attempts in the past to create within-strain differences in cognitive ability in order to come to a better understanding of how cognition is coded within the genome. For example, Tyron (Tyron, 1931) claimed that he had 'created' two strains (maze bright and maze dull) of rat that differed in 'a fairly general ability to learn'. However, it is clear that the Tryon strains do not differ in the fundamental mechanisms underlying learning and memory formation but instead differ in motivation, social dominance and aggression. These differences may explain the difference in learning in Tyron's maze experiments. More recently, similar conclusions could be drawn from *Syracuse* rats in that the strain-specific purported cognitive differences are really differences in variables related to the emotional and affective domains (Brush, 2003). Today, researchers take a different, more successful, path in attempting to understand the genomic underpinnings of cognitive ability by using specific mutations in the mouse and fly to determine the role played by specific genes (Dukas, 2008; Nguyen, 2006; Tully, 1996).

However, not withstanding the above arguments, there are strain differences within the same species as regards the ability to learn and form LTM. For example, laboratory bumble bees were trained to overcome an innate preference for blue and learn to associate a yellow colour as a predictor of floral reward. Bumble bees learn and remember this task, but individuals and colonies vary in their speed and accuracy with this new skill (Chittka et al., 2004; Raine et al., 2006). The task is ecologically relevant because foraging bees use a variety of cues, including colour and scent, to recognize and learn the flowers from which they collect food (Menzel 1985; Scheiner et al., 2001; Chittka and Raine 2006). Raine and Chittka (Raine and Chittka, 2008) go even further and show that the variation in learning speed between different bumble bee colonies is directly correlated with foraging performance. Colonies vary in learning speed by a factor of nearly five, with the slowest learning colonies collecting 40% less nectar than the fastest learning colonies. Such

a difference is suggestive of strong selection for higher learning speed. That is, hives that have faster and better learners make more honey and have a better chance of survival.

While it is intuitively appealing to assume that variation in learning and memory-forming ability is adaptive (Johnston, 1982; Dukas, 1998), few studies have yet been conducted to specifically examine this link under natural conditions. It is for this reason that we set out to determine whether the strain differences between the Jackson and Belly snails that we saw in the laboratory would continue to be observed in the wild, both at the home and at the visiting pond. As mentioned above, within the exact same strain cognate differences are seen that are laboratory dependent (Crabbe et al., 1999; Crabbe et al., 2005; Wahlsten et al., 2006). We saw here that the strain differences in memory-forming ability were not changed when snails were trained in the wild whether they were at their home pond or a visiting pond. Thus, we can reject the hypothesis that the difference in LTM-forming ability between these two strains is the result of differing abilities of the two strains to adapt to laboratory conditions. We can also rule out differences in the training techniques of the researchers as similar data were collected by the four snail trainers (M.O., K.H., K.L. and J.H.). Finally, the difference between Belly and Jackson snails as regards memory-forming capability was not the result of one strain being trained by one investigator and the other strain being trained by the other investigator. Approximately equal numbers of snails from each pond were trained by each investigator blindly in the lab setting.

We also believe that we can rule out the possibility that there are significant differences in learning ability between the two strains. Our reasoning is as follows. While learning and memory are related they are two different processes each with their own rules and underlying mechanisms (Milner et al., 1998). In *L. stagnalis* there are at least two different forms of long-lasting memory seen following learning: ITM and LTM (Lukowiak et al., 2000; Lukowiak et al., 2003b; Sangha et al., 2003a; Sangha et al., 2003b; Sangha et al., 2003c; Sangha et al., 2003d; Sangha et al., 2003e; Smyth et al., 2003; Parvez et al., 2006; Martens et al., 2007a; Lattal et al., 2007). ITM is dependent on new protein synthesis whilst LTM is dependent on both new protein synthesis and altered gene activity (Sangha et al., 2003a). In our data we saw that the two strains were equally competent to form ITM. This implies that the two strains: (1) can learn (i.e. the acquisition of a new skill); (2) can form ITM (a memory that lasts about 3 h); but (3) have differences in the ability to alter gene activity and new protein synthesis in neurons, such as RPeD1, which are necessary for LTM formation (Scheibenstock et al., 2002; Spencer et al., 2002). We will in future experiments examine whether there are strain-related differences in RPeD1 electrophysiological activity following training, both 3 h (ITM present in both) and 24 h (LTM only in Belly snails) later. It is possible that we will see differences in RPeD1 activity at 3 h between the two strains as the activity in the Belly snails may be different due the encoding of LTM, even though the behaviour phenotypes (i.e. ITM) are similar. We also expect to find differences in neuronal activity at 24 h. If we find that there are these expected differences we will begin to perform molecular experiments examining whether there are differences, for example, in the number of mRNA copies of the differing CREB1 *vs* CREB2 (the activator and suppressive transcription factors) whose ratio has been hypothesized to play a major role in determining whether LTM will be formed (Sadamoto et al., 2003; Azami et al., 2006; Sugai et al., 2006; Sugai et al., 2007) in *Lymnaea*.

It is intriguing to us that there are these strain-related cognitive differences between the two populations of *Lymnaea* and we are attempting to determine the reason for the difference. One obvious difference between the two populations is hydro-physical. The Jackson snails come from an isolated man-made 20 year old dug-out that has no physical connection with any other body of water, whereas the Belly snails come from a natural series of interconnected ponds that undergo seasonal flooding in the spring with the melting of the winter snow pack and spring rains. We know that the owner of the Jackson pond did not purposely introduce *Lymnaea* to the pond and we presume that the snails are descended from one or more snails that were introduced by visiting waterfowl. Whether a 'genetic bottle-neck' phenomenon is the reason for the difference in cognitive ability remains to be determined. That is, in every cohort of snails that we have trained over some 15 years there are always some snails that just do not form LTM with any of our training procedures (Lukowiak et al., 2003a). If the founding member(s) of the Jackson pond was a snail(s) that was 'memory challenged' then the off-spring might also be 'memory challenged' as it appears that the ability to form LTM is a heritable characteristic in *Lymnaea* (Orr et al., 2008).

Another possible reason for the difference in LTM-forming ability is the presence of parasites (e.g. Trematodes; schistosomes in Alberta ponds that cause 'swimmers itch'; personal experience; K.W.) in the snail. We found that over 90% of the Jackson snails examined were infected with parasites, whilst only approximately 10% of Belly snails were parasitized. However, we found that parasitized Belly snails were capable of forming LTM to the same extent as their non-parasitized pond mates. Further, as shown here, a few individual Jackson snails that are parasitized are capable of forming enhanced LTM, too. It may be, however, that because of the heavy parasitic load in the Jackson pond this somehow alters the ability of the majority of snails to initiate the necessary genomic response following training to form LTM in neurons such as RPeD1. We have already demonstrated that an environmental stressor, crowding, selectively blocks LTM formation in snails without altering the ability to learn and form ITM (de Caigny and Lukowiak, 2008a; de Caigny and Lukowiak, 2008b). Thus, crowding acts primarily on genomic activity as we suggest the heavy parasitic load does. Future experiments will attempt to elucidate whether the stress associated with a heavy parasitic load is the reason why the Jackson *Lymnaea* have difficulty in forming LTM. In this regard it is appropriate to cite Gregear and colleagues (Gregear et al., 2006), who demonstrated that a parasitic infection in bumble bees resulted in changes in cognitive function possibly as a result of the interaction between the immune and nervous systems. It is also true that snails in 'stand alone' small ponds such as the Jackson pond typically are more highly parasitized than those in ponds or lakes that are larger and 'connected' (Voutilainen et al., 2008).

We are uncertain whether there is an 'ecological' advantage to snails that form a 'better' memory. That is, we have no evidence that Belly snails are more or less 'fit' than Jackson snails. We have previously hypothesized that the enhanced ability to form memory following exposure of snails to the scent of a predator (Orr and Lukowiak., 2008) should qualify as an adaptive anti-predator response. We have recently shown that exposure of Jackson snails to the scent of a sympatric predator [tiger salamander; salamander effluent, SE (Orr et al., 2009)] significantly enhances their ability to form LTM. We did not see any enhancement of memory in the Belly snails following exposure to SE, but this is most likely due to a 'ceiling effect' as their memory is so good to begin with. However, the Belly and Jackson snails show similar alterations in aerial respiratory behaviour when they detect the presence of a sympatric predator. They both significantly decrease their total breathing time

in a hypoxic situation (Orr et al., 2009). Thus, while there may be differences between these strains as regards cognitive ability, they respond similarly to the presence of a predator. Finally, we are again humbled by our good fortune in having initially sampled Belly snails for their ability to form LTM. If we had only sampled Jackson snails we would have concluded that there were no differences in memory-forming ability between Dutch (both wild and laboratory-reared) and Alberta snails and might not have attempted to 'enrich' the laboratory environment by the introduction of the scent of a predator (Orr et al., 2007; Orr et al., 2008; Orr and Lukowiak, 2008). We are also attempting to determine why the Belly snails are so good at forming LTM. We are uncertain whether they are also better at forming LTM with different tasks (e.g. appetitive or aversive food conditioning) and these experiments are underway. We are also determining whether juvenile Belly snails are capable of forming LTM as we have previously shown that juvenile lab-reared snails do not form LTM (McComb et al., 2003; McComb et al., 2005).

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REFERENCES

Azami, S., Wagatsuma, A., Sadamoto, H., Hatakeyama, D., Usami, T., Fujie, M., Koyanagi, R., Azumi, K., Fujito, Y., Lukowiak, K. and Ito, E. (2006). Altered gene activity correlated with long-term memory formation of conditioned taste aversion in Lymnaea. *J. Neuro. Res.* **84**, 1610-1620.

Boag, D. A. and Pearlstone, P. S. M. (1979). Life-cycle of *Lymnaea stagnalis* (Pulmonata, Gastropoda) in Southwestern Alberta. *Can. J. Zool.* **57**, 353-362.

Boag, D. A., Thomson, C. and Vanes, J. (1984). Vertical-distribution of young pond snails (Basommatophora, Pulmonata) – Implications for survival. *Can. J. Zool.* **62**, 1485-1490.

Brush, F. R., Gendron, C. M. and Isaacson, M. D. (1999). A selective genetic analysis of the Syracuse high- and low-avoidance (SHA/Bru and SLA/Bru) strains of rats (*Rattus norvegicus*). *Behav. Brain Res.* **106**, 1-11.

Chittka, L. and Raine, N. (2006). Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**, 428-435.

Chittka, L., Ings, T. C. and Raine, N. E. (2004). Chance and adaptation in the evolution of island bumblebee behaviour. *Popul. Ecol.* **46**, 243-251.

Clarke, A. H. (1981). *The Freshwater Molluscs of Canada*. Ottawa: Museum of Natural History.

Clifford, H. F. (1991). *Aquatic Invertebrates Of Alberta*. Edmonton: University of Alberta Press.

Crabbe, J. C., Wahlsten, D. and Dudai, B. C. (1999). Genetics of mouse behavior: interactions with laboratory environment. *Science* **284**, 1670-1672.

Crabbe, J. C., Metten, P., Cameron, A. J. and Wahlsten, D. (2005). An analysis of the genetics of alcohol intoxication in inbred mice. *Neurosci. Biobehav. Rev.* **28**, 785-802.

de Caigny, P. and Lukowiak, K. (2008a). Crowding, an environmental stressor, blocks long-term memory formation in *Lymnaea*. *J. Exp. Biol.* **211**, 2678-2688.

de Caigny, P. and Lukowiak, K. (2008b). A clash of stressors and LTM formation. *Commun. Integr. Biol.* **2**, 1-3.

Dukas, R. (2008). Evolutionary biology of insect learning. *Annu. Rev. Entomol.* **53**, 145-160.

Dukas, R. (1998). *Cognitive Ecology: The Evolutionary Ecology Of Information Processing And Decision Making*. Chicago, IL: University of Chicago Press.

Gittenberger, E., Groenenberg, D., Kokshoorn, B. and Preece, R. (2006). Molecular trails from hitch-hiking snails. *Nature* **439**, 409.

Gregear, R., Otterstatter, M. and Thomson, J. (2006). Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proc. R. Soc. Lond. B.* **273**, 1073-1078.

Haney, J. and Lukowiak, K. (2001). Context learning and the effect of context on memory retrieval in *Lymnaea*. *Learn. Mem.* **8**, 35-43.

Harker, K. T. and Wishaw, I. (2002). Place and matching-to-place spatial learning affected by rat inbreeding (Dark/Agouti, Fischer 344) and albinism (Wistar, Sprague-Dawley) but not domestication (wild rat vs. Long-Evans, Fischer-Norway). *Behav. Brain Res.* **134**, 467-477.

Ings, T., Schikora, J. and Chittka, L. (2005). Bumblebees, humble pollinators or assiduous invaders? A population comparison of foraging performance in *Bombus terrestris*. *Oceologia* **144**, 508-516.

Inoue, T., Takaski, K., Lukowiak, K. and Syed, N. (1996). Inhibition of the respiratory pattern-generating neurons by an identified whole body withdrawal interneuron of *Lymnaea stagnalis*. *J. Exp. Biol.* **199**, 1887-1898.

Johnston, T. D. (1982). Selective costs and benefits in the evolution of learning. *Adv. Study Behav.* **12**, 65-106.

Lattal, K. M., Radulovic, J. and Lukowiak, K. (2006). Extinction: does it or doesn't it? The requirement of altered gene activity and new protein synthesis. *Biol. Psychiatry* **60**, 344-351.

Lukowiak, K., Ringseis, E., Spencer, G., Wildering, W. and Syed, N. (1996). Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **199**, 683-691.

Lukowiak, K., Cotter, R., Westley, J., Ringseis, E., Spencer, G. and Syed, N. (1998). Long term memory of an operantly conditioned respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **201**, 877-882.

Lukowiak, K., Adatia, A., Krygier, D. and Syed, N. (2000). Operant conditioning in *Lymnaea*: Evidence for intermediate and long-term memory. *Learn. Mem.* **7**, 140-150.

Lukowiak, K., Sangha, S., McComb, C., Varshney, N., Rosengger, D., Sadamoto, H. and Scheibenstock, A. (2003a). Associative learning, long-term memory, and the assignment of 'marks' in the pond snail, *Lymnaea*. *J. Exp. Biol.* **206**, 2097-2103.

Lukowiak, K., Haque, Z., Spencer, G., Varshney, N., Sangha, S. and Syed, N. (2003b). Long-term memory survives nerve injury and the subsequent regeneration process. *Learn. Mem.* **10**, 44-54.

Lukowiak, K., Martens, K., Rosenegger, D., Browning, K., de Caigny, P. and Orr, M. (2008). The perception of stress alters adaptive behaviours in *Lymnaea stagnalis* *J. Exp. Biol.* **211**, 1747-1756.

Martens, K., Amarell, M., Parvez, K., Hittel, K., De Caigny, P., Ito, E. and Lukowiak, K. (2007a). One-trial conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* **88**, 232-242.

Martens, K. R., De Caigny, P., Parvez, K., Amarell, M., Wong, C. and Lukowiak, K. (2007b). Stressful stimuli modulate memory formation in *Lymnaea stagnalis*. *Neurobiol. Learn Mem.* **87**, 391-403.

McComb, C., Meems, R., Syed, N. and Lukowiak, K. (2003). Electrophysiological differences in the neuronal circuit controlling aerial respiratory behaviour between juvenile and adult *Lymnaea*. *J. Neurophysiol.* **90**, 983-992.

McComb, C., Varshney, N. and Lukowiak, K. (2005). Juvenile *Lymnaea* ventilate, learn and remember differently than do adult *Lymnaea*. *J. Exp. Biol.* **208**, 1459-1467.

Menzel, R. (1985). Learning in honey bees in an ecological and behavioral context. In *Experimental Behavioral Ecology* (ed. B. Holldobler and M. Lindauer), pp. 55-74. Stuttgart, Germany: Gustav Fischer Verlag.

Milner, B., Squire, L. R. and Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron* **20**, 445-468.

Nguyen, P. V. (2006). Comparative plasticity of brain synapses in inbred mouse strains. *J. Exp. Biol.* **209**, 2293-2303.

Orr, M. and Lukowiak, K. (2010). Sympatric predator detection alters cutaneous respiration in *Lymnaea*. *Commun. Integr. Biol.* (in Press).

Orr, M. and Lukowiak, K. (2008).Electrophysiological and behavioral evidence demonstrating that predator detection alters adaptive behaviors in the snail *Lymnaea*. *J. Neurosci.* **28**, 2726-2734.

Orr, M., El-Bekai, M., Lui, M., Watson, K. and Lukowiak, K. (2007). Predator detection in *Lymnaea stagnalis*. *J. Exp. Biol.* **210**, 4150-4158.

Orr, M., Hittel, K. and Lukowiak, K. (2008). Comparing memory-forming capabilities between laboratory-reared and wild *Lymnaea*. *J. Exp. Biol.* **211**, 2807-2816.

Orr, M., Hittel, K. and Lukowiak, K. (2009). 'Different strokes for different folks': geographically isolated strains of *Lymnaea stagnalis* only respond to sympatric predators and have different memory forming capabilities. *J. Exp. Biol.* **212**, 2237-2247.

Parvez, K., Moisseev, V. and Lukowiak, K. (2006). A context specific single contingent-reinforcing stimulus boosts intermediate-term memory into long-term memory. *Eur. J. Neurosci.* **24**, 606-616.

Raine, N. and Chittka, L. (2008). The correlation of learning speed and natural foraging success in bumble-bees. *Proc R. Soc. Lond. B* **275**, 803-808.

Raine, N. E., Ings, T. C., Ramos-Rodrıguez, O. and Chittka, L. (2006). Inter-colony variation in learning performance of a wild British bumblebee population (Hymenoptera: Apidae: *Bombus terrestris audax*). *Entomol. Gen.* **28**, 241-256.

Rosenegger, D., Roth, S. and Lukowiak, K. (2004). Learning and memory in *Lymnaea* are negatively altered by acute low-level concentrations of hydrogen sulphide. *J. Exp. Biol.* **207**, 2621-2630.

Sadamoto, H., Sato, H., Kobayashi, S., Murakami, J., Aonuma, H., Ando, H., Fujito, Y., Hamano, K., Awaji, M., Lukowiak, K., Urano, A. and Ito, E. (2004). CREB in the pond snail *Lymnaea stagnalis*: Cloning, gene expression and function in identifiable neurons of the central nervous system. *J. Neurobiol.* **58**, 455-466.

Sangha, S., Scheibenstock, A., McComb, C. and Lukowiak, K. (2003a). Intermediate and long-term memories of associative learning are differentially affected by transcription *vs* translation blockers in *Lymnaea*. *J. Exp. Biol.* **206**, 1605-1613.

Sangha, S., McComb, C. and Lukowiak, K. (2003b). Forgetting and the extension of memory in *Lymnaea*. *J. Exp. Biol.* **206**, 71-77.

Sangha, S., Morrow, R., Smyth, K., Cooke, R. and Lukowiak, K. (2003c). Cooling blocks ITM and LTM formation and preserves memory. *Neurobiol. Learn. Mem.* **80**, 130-139.

Sangha, S., Scheibenstock, A. and Lukowiak, K. (2003d). Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of RPeD1. *J. Neurosci.* **23**, 8034-8040.

Sangha, S., Scheibenstock, A., Morrow, R. and Lukowiak, K. (2003e). Extinction requires new RNA and protein synthesis and the soma of the cell RPeD1 in *Lymnaea stagnalis*. *J. Neurosci.* **23**, 9842-9851.

Scheibenstock, A., Krygier, D., Haque, Z., Syed, N. and Lukowiak, K. (2002). The soma of RPeD1 must be present for LTM formation of associative learning in Lymnaea. *J. Neurophysiol.* **88**, 1584-1591.

Scheiner, R., Page, R., Jr and Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Neurobiol. Learn. Mem.* **76**, 138-150.

Smyth, K., Sangha, S. and Lukowiak, K. (2002). Gone but not forgotten: The lingering effects of intermediate term memory on the persistence of LTM. *J. Exp. Biol.* **205**, 131-140.

Spencer, G. E., Kazmi, M. H., Syed, N. I. and Lukowiak, K. (2002). Changes in the activity of a CPG neuron after the reinforcement of an operantly conditioned behavior in *Lymnaea*. *J. Neurophysiol.* **88**, 1915-1923.

Sugai, R., Shiga, H., Azami, S., Watanabe, T., Fujito, Y., Lukowiak, K. and Ito, E. (2006). Taste discrimination in conditioned taste aversion of the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **209**, 826-833.

Sugai, R., Azami, S., Shiga, H., Watanabe, T., Sadamoto, H., Kobayashi, S., Hatakeyama, D., Fujito, Y., Lukowiak, K. and Ito, E. (2007). One-trial conditioned taste aversion in *Lymnaea*: good and poor performers in long-term memory acquisition. *J. Exp. Biol.* **210**, 1225-1237.

Syed, N. I., Bulloch, A. G. M. and Lukowiak, K. (1990). In vitro reconstruction of the respiratory central pattern generator of the mollusk *Lymnaea*. *Science* **250**, 282-285.

Syed, N. I., Ridgway, R., Lukowiak, K. and Bulloch, A. G. M. (1992). Transplantation and functional integration of an identified respiratory interneuron in *Lymnaea stagnalis*. *Neuron* **8**, 767-774.

Tolman, E. C. (1924). The inheritance of maze-learning ability in rats. *J. Comp. Psychol.* **4**, 1-18.

Tryon, R. C. (1931b). Studies in individual differences in maze ability. III. The community of function between two maze abilities. *J. Comp. Psychol.* **12**, 95-116.

Tully, T. (1996). Discovery of genes involved with learning and memory: An experimental synthesis of Hirschian and Benzerian perspectives. *Proc. Natl. Acad. Sci. USA* **93**, 13460-13467.

Voutilainen, A., van Ooik, T., Puurtinen, M., Kortet, R. and Taskinen, J. (2009). Relationship between prevalence of trematode parasite *Diplostomum* sp. and population density of its snail host *Lymnaea stagnalis* in lakes and ponds of Finland. *Aquat. Ecol.* **43**, 351-357.

Wahlsten, D., Bachmanov, A., Finn, D. and Crabbe, J. (2006). Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. *Proc. Natl. Acad. Sci. USA* **103**, 16364-16369.